

# Induced changes in the affinity of 1,25-dihydroxyvitamin D<sub>3</sub> receptors in chick intestine

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The contents of 1,25-dihydroxyvitamin D<sub>3</sub> (1,25(OH)<sub>2</sub>D<sub>3</sub>) in plasma and intestinal mucosa were increased by dietary calcium and by dietary phosphorus restriction. The concentration of intestinal occupied receptors for 1,25(OH)<sub>2</sub>D<sub>3</sub> was higher in calcium-restricted birds. The affinity (association constant) of intestinal receptors for 1,25(OH)<sub>2</sub>D<sub>3</sub> was lower in phosphorus-restricted chicks, as compared to control or calcium-restricted chicks. The number of binding sites were not influenced by dietary calcium or phosphorus restriction.

Chick	P-depletion	Intestine	1,25-Dihydroxyvitamin D <sub>3</sub>	Receptor	Affinity
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## 1. INTRODUCTION

The physiological response to hormones is dependent on the hormone concentration, and the affinity and number of receptors on the target cells. Although the dependence of the response on hormone concentration has been clearly established for many hormones, including parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D<sub>3</sub> (1,25(OH)<sub>2</sub>D<sub>3</sub>) [1–4], regulation at the level of the receptors has been described in only a few cases. For example, PTH receptors level in the kidney declined during calcium or vitamin D deficiencies [5] and the concentration of intestinal 1,25(OH)<sub>2</sub>D<sub>3</sub> receptors changed during neonatal development and declined as a result of glucocorticoids treatment [6–8]. Regulation in vivo of the affinity of steroid hormones to their receptors has been described in a few cases [9], but not with regard to 1,25(OH)<sub>2</sub>D<sub>3</sub> receptors.

In the present study, we demonstrated a decline in the affinity (association constant) of 1,25-(OH)<sub>2</sub>D<sub>3</sub> receptors as a result of phosphorus deficiency. In the chicken this dietary treatment was previously shown to affect the hormone binding [10,11] to intestinal receptors.

## 2. EXPERIMENTAL

One-day-old male chicks (White Rock) were raised on a complete diet containing 1.1 and 0.7% calcium (Ca) and phosphorus (P). At the age of 10 days, the birds were divided into three lots which were fed for 9 days a Ca-deficient (0.2%), a P-deficient (0.35%), or the same diet (control). At the end of the feeding period, heparinized blood samples were taken for the determination of calcium [12], inorganic phosphorus (P<sub>i</sub>) [12] and 1,25(OH)<sub>2</sub>D<sub>3</sub>. Intestinal chromatin was prepared in a low KCl buffer and the occupied receptors for 1,25(OH)<sub>2</sub>D<sub>3</sub> were assessed as in [10].

The association constants ( $K_A$ ) were calculated from the Scatchard [13] analysis of 1,25(OH)<sub>2</sub>D<sub>3</sub> specific binding to the chromatin fraction. For this assay, 0.3 ml suspension (20%, w/v) was equilibrated for 18 h at 4°C with 1,25(OH)<sub>2</sub>D<sub>3</sub>[25,27-<sup>3</sup>H] (0.1–6.4 M × 10<sup>−9</sup>). Bound 1,25(OH)<sub>2</sub>D<sub>3</sub> was separated by the use of hydroxylapatite [14]. Non-specific binding was defined as that resistant to a 200-fold excess of unlabeled 1,25(OH)<sub>2</sub>D<sub>3</sub>.

Blood plasma or intestinal chromatin was extracted [15], dried under nitrogen, dissolved in 1 ml hexane/diethyl ether (2:1, v/v), filtered

Table 1

Changes in the affinity and concentration of the intestinal receptor for 1,25(OH)<sub>2</sub>D<sub>3</sub> in calcium- or phosphorus-deficient chicks

Diet	Control	Low-Ca	Low-P
Plasma Ca, mg/dl	10.3 ± 0.3 <sup>a</sup>	8.7 ± 0.2 <sup>b</sup>	12.5 ± 0.7 <sup>c</sup>
Plasma P <sub>i</sub> , mg/dl	5.6 ± 0.2 <sup>a</sup>	4.4 ± 0.6 <sup>a</sup>	2.6 ± 0.2 <sup>b</sup>
Plasma 1,25(OH) D, pmol/ml	0.36 ± 0.03 <sup>a</sup>	0.71 ± 0.06 <sup>b</sup>	0.56 ± 0.02 <sup>b</sup>
1,25(OH) D intestinal chromatin, pmol/g	1.51 ± 0.08 <sup>a</sup>	2.35 ± 0.21 <sup>b</sup>	2.25 ± 0.11 <sup>b</sup>
Intestinal occupied receptor, pmol/g	1.20 ± 0.20 <sup>a</sup>	2.21 ± 0.27 <sup>b</sup>	1.57 ± 0.16 <sup>ab</sup>
Association constant (K <sub>A</sub> ), 10 <sup>9</sup> M <sup>-1</sup> d,e	1.16 ± 0.20 <sup>a</sup>	1.09 ± 0.20 <sup>a</sup>	0.67 ± 0.21 <sup>b</sup>
Binding sites (N <sub>max</sub> ) nmol/mg <sup>d</sup>	0.53	0.66	0.62

<sup>a-c</sup> Mean ± SEM of 7 or 8 chicks. Means designated by different letters are significantly different ( $p < 0.01$ )

<sup>d</sup> Values obtained from the Scatchard analysis (fig.1)

<sup>e</sup> Corresponds with equilibrium dissociation constant ( $K_D = \frac{1}{K_A}$ ) of 0.86, 0.97 and 1.52 nM, respectively

through Sep Pak silica cartridge (Waters, Milford MA) and then eluted by 9 ml of the same solvent mixture. The retained 1,25(OH)<sub>2</sub>D<sub>3</sub> was eluted from the Sep Pak cartridge by 12 ml of 5% Metanol in diethyl ether. The recovery of 1,25(OH)<sub>2</sub>D<sub>3</sub> after this step of purification was  $99 \pm 1\%$ .

The samples were then filtered through a 0.5- $\mu$ m Teflon filter (Millipore, Bedford MA) and chromatographed by HPLC (Waters) with a System Controller (Model 720), attached to a fraction collector. The samples were eluted at a flow-rate of 4 ml/min through a 10 × 0.8 cm Radial-Pak  $\mu$ Porasil cartridge and a Z-module Radial Compression Separation System (Waters), using an isocratic-gradient combination of solvents from 5–20% isopropanol in hexane. Fractions containing the peak regions of the various D<sub>3</sub> metabolites were collected. The concentration of 1,25(OH)<sub>2</sub>D<sub>3</sub> was determined by a competitive binding assay [16] except that the cytosol was prepared in the presence of sodium molybdate [17]. Protein was determined by using Bio-Rad (Richmond CA) protein assay [17].

### 3. RESULTS

The results (table 1) show, as expected, a hypocalcemia in the Ca-deficient chicks and a hypercalcemia-hypophosphatemia in the P-deficient chicks. 1,25(OH)<sub>2</sub>D<sub>3</sub> in the plasma or in the chromatin fraction was significantly higher

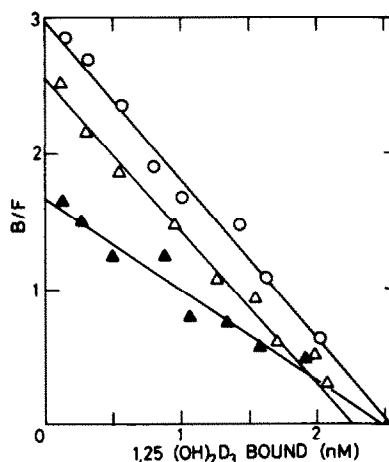


Fig.1. Scatchard analysis of 1,25(OH)<sub>2</sub>D<sub>3</sub> specific binding to chick intestinal crude chromatin, obtained from control (○), calcium-restricted (Δ), or phosphorus-restricted chicks (▲). Suspension of fresh chromatin (0.3 ml, 4–5 mg protein/ml) was equilibrated with 1,25(OH)<sub>2</sub>D<sub>3</sub>[26,27-<sup>3</sup>H] (0.1–6.4 mM) at 4°C for 18 h. Bound 1,25(OH)<sub>2</sub>D<sub>3</sub> was measured through the use of hydroxylapatite. Non-specific binding was defined as that resistant to a 200-fold excess of unlabeled 1,25(OH)<sub>2</sub>D<sub>3</sub>. The association constants (K<sub>A</sub>), as calculated from the slope, and the number of specific binding sites, as estimated from the x-intercept, are given in table 1. Values represent the means of triplicate determinations. A covariance analysis indicated that the slopes obtained for calcium-restricted or control birds were not significantly different, while the slope of the phosphorus-deficient birds was significantly different from control or phosphorus-deficient birds.

( $p < 0.01$ ) in the Ca- or in the P-deficient, as compared with the control birds.

Occupied  $1,25(\text{OH})_2\text{D}_3$  receptors of the chromatin fraction measured by the exchange assay, were significantly higher ( $p < 0.01$ ) only in the intestine of Ca-deficient birds, whereas there was no significant difference between the P-deficient and the control birds ( $p > 0.05$ ), despite the significant difference between the groups in the concentration of  $1,25(\text{OH})_2\text{D}_3$  in the chromatin.

The equilibrium constant ( $K_A$ ) and the number of binding sites ( $N_{\text{max}}$ ) in the intestinal chromatin (20%, w/v), prepared from control, Ca- or P-deficient chicks, were estimated from the slope and  $x$ -intercept, respectively, of the Scatchard plot (fig.1), and are given in table 1. Covariance analysis suggested that the association constants obtained for chromatin of control or Ca-deficient birds were not significantly different ( $p > 0.05$ ).

The association constant was significantly ( $p < 0.01$ ) lower in P-deficient birds than that of the control or calcium-deficient ones. There was no significant difference between the two latter groups. The  $N_{\text{max}}$  estimated from the  $x$ -intercepts did not differ in the chromatin taken from the different groups.

#### 4. DISCUSSION

Two approaches have been used in order to characterize the kinetics of intestinal  $1,25(\text{OH})_2\text{D}_3$  receptors. The first was based on the determination of 'occupied receptors' by an exchange assay [10]. The values of the occupied receptors, as determined by this assay, represent products of the number of sites, their affinity, and the hormone concentration in vivo [18]. The other approach, based on the Scatchard analysis [13], determines the number of receptor sites ( $N_{\text{max}}$ ) and their affinity ( $K_A$ ).

The contents of occupied receptors was lower in the chromatin prepared from P-deficient than from Ca-deficient birds (table 1), despite the similar contents of  $1,25(\text{OH})_2\text{D}_3$  measured in the same chromatin samples. This suggested a lower affinity or number of sites in the P-deficient bird's intestinal mucosa.

Using the Scatchard analysis, a lower affinity of intestinal receptors for  $1,25(\text{OH})_2\text{D}_3$  was observed in P-deficient birds as compared with control or

Ca-deficient ones. The number of receptor sites per mg protein was not changed significantly.

The molecular mechanism of the reduced affinity of the receptors from P-deficient birds is still not clear. The lower affinity of the receptors in P-deficient birds could be a result of homeostatic mechanism aimed at protecting the birds from the undesirable increase in Ca absorption [19] in P deficiency conditions which leads to hypercalcemia, poor bone formation, and kidney damage [20].

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